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# An Attempt to Identify the Origin of Pinus brutia Ten. Plantations in Israel by Needle Resin Composition[1])


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### Abstract

Identification of seed sources of plantations of *Pinus brutia* TEN. subsp. *brutia* from imported seed, was attempted by determination of the needle resin composition. The results were compared with the resin composition of various identified seed sources in provenance trial plots. The seed sources present in trial plots were grouped by cluster and discriminant analysis procedures mainly into low -and high -altitude western Anatolian groups and an eastern Anatolian group, with the Iraq, Cyprus and Crete provenances occupying isolated positions.

There is reason to believe that plantations established in the 1930s and 1940s were of Greek origin, whereas some of those established in the 1960s and 1970s were in part of Turkish origin.



*Key words: Pinus brutia,* resin composition, provenances.


### Introduction

The range of *Pinus brutia* TEN. subsp. *brutia,* the eastern vicariad of *Pinus halepensis* MILL., extends from the Greece Aegen Islands through Turkey to Lebanon and northern Iraq (CRITCHFIELD and LITTLE, 1966). The subspecies was introduced into Israel in the late 1920s (HETH, 1968), but only recentely it is widely used in afforestation projects; at present, out of an area of man-made conifer forests of approximately 60,000 ha, *P. brutia* plantations extends over some 10,000 ha. Reliable knowledge on the seed origin of the plantations is almost nonexistent. According to available data, most of the seed used in the last 40 or 50 years appears to have been imported from Greece, Cyprus and Turkey, but locally collected second-generation seed is being used increasingly (Y. REVES, personal communication). Seed was imported at a rate of 10 kg to 20 kg each year, which means that the seed crop of at least 70 trees was used here yearly in the reforestation projects. It is therefore of interest to identify, if possible, seed sources well adapted to local conditions in order to utilize more fully the potential for growth of brutia pine, since the tree is clearly superior to the still widely used Aleppo pine (*P. halepensis*) owing to the straight shape of the bole and its resistance to the pine bast scale *Matsucoccus josephi* BODENH. et HARPAZ, the major (and often lethal) pest of *P. halepensis* (MENDEL, 1984).

Determinations were made of the needle resin composition in local plantations of *Pinus brutia* TEN.; the results were compared with the resin composition determined in trees from various registered seed sources growing in provenance trial plots established in 1976 in conjunction with the IUFRO-FAO project 4 bis. This method was chosen because analysis of monoterpene composition was shown to be useful for assessing possible geographic origins (SQUILLACE et al., 1980).

To sample a large number of trees and obtain statistically valid results, it proved expedient to investigate the resin composition of needles rather than of the cortex or xylem, although this involved a repeat analysis of various seed origins in the provenance trials (SCHILLER and GRUNWALD, 1987).

### Materials and Methods

*1. Plant material*

Needle samples were collected in the autumn from all the trees growing in trial plots planted in 1976 at 3 sites: on Mt. Carmel at Ramat ha'Nadiv (32°32'N, 34°56'E); in the Judean Foothills near Nahshon (31°50'N, 34°58'E); and in the Judean Mountains at ha' Qedoshim forest (31°47'N, 35°05'E). The 14 provenances under trial at the three locations are shown in *table 1* and *figure 1.* The total number of trees sampled was 232, with 7 to 20 trees per provenance.

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Sampling was extended subsequently to 20 stands (about 30 trees per stand) of unknown seed origin and different ages in 8 forests. Ten stands and 4 stands planted in different years were sampled in the Ben Shemen and Mevo Betar plantations, respectively; one stand each was sampled in the other plantations (*Table 1 bis*). The number of trees sampled was 517.

The sampling was carried out in the autumn within 2 month, to prevent as much as possible differences in the resin composition due to climatic or needle age influence, since the degree of stability of the resin composition of *P. brutia* during the growing season was not known.

*2. Resin analysis*

Current year fully grown, healthy and physiologically active needles, at a rate of 200 g f.wt. per tree, were used for chemical analysis, the foliage from each tree being sampled and analyzed separately. The resin (0.1 ml to 0.5 ml per sample) was extracted by steam distillation. From each sample 1.5 $\mu$l was analyzed with a Packard 7400 gas-liquid chromatograph fitted with a flame ionization detector and a glass column 4 m in length and 2 mm in diameter, packed with OV—101 (100% methyl fluid) 8% on chromosorb W-60/80. Operating conditions were 250° C at the injector and detector and 50° C to 230° C at the column, with the temperature rising at a rate of 5° C $min^{-1}$. Nitrogen was used as the carrier gas at a flow rate of 25 ml $min^{-1}$.

Peak area on the chromatograms, *i.e.*, resin compounds (*Fig. 2*), was calculated as component percentages of the sum of all peaks with a 3390-A Hewlett -Packard integrator; for statistical analysis the percentages were transformed using the logarithmic transformation Y= Ln (Xi) as recommended by Kung (1988) to achieve approximate homogeneity of the variances. The data obtained were analyzed by discriminant and cluster procedures included in the SAS programs package (SAS, 1985). Discriminant analysis was performed in wo stages. First backward stepwise discriminant procedure was carried out in order to reduce the set of compounds to those which contributed significantly to the discrimination. When the set of compounds most suitable for discrimination was chosen, each observation was classified into one of the provenances by calculating its discrimination function or Mahalanobis distance from every group mean or centroid and classified it to the group whose centroid was nearest or equivalently, to the group with highest posterior probability of membership. The set of compound values classified to a certain provenance will be said to represent the "chemotype" of the provenance. In cluster analysis by average linkage methods the generalized distances were calculated using all 36 compounds to allow the whole resin profile to determine the cluster formations.

*Table 1.* — Seed origin of Pinus brutia subsp. brutia in provenance trials in Israel.

| IUFRO No. | Country | Provenance | Lat. N (° ') | Long. E (° ') | Alt. (m) |
|---|---|---|---|---|---|
| $B_1$ | Greece | Chania, Crete | 35 17 | 23 57 | 300. |
| $B_3$ | Greece | Lussithiou, Crete | 35 06 | 25 37 | 1100 |
| $B_5$ | Cyprus | | 35 08 | 33 17 | 150 |
| $B_6$ | Turkey | Marmaris | 37 00 | 28 18 | 175. |
| $B_7$ | Turkey | Isparta | 38 04 | 29 32 | 1100. |
| $B_8$ | Turkey | Duzlercani | 37 03 | 30 25 | 175. |
| $B_9$ | Turkey | Pamucak | 37 40 | 30 41 | 1000 |
| $B_{10}$ | Turkey | Bozburum | 37 21 | 30 45 | 500. |
| $B_{11}$ | Turkey | Bakara | 36 29 | 32 43 | 1300. |
| $B_{12}$ | Turkey | Silifke | 36 13 | 33 43 | 100. |
| $B_{13}$ | Turkey | Gamgolu | 41 50 | 35 20 | 70. |
| $B_{14}$ | Turkey | Baspinar | 37 48 | 35 15 | 700 |
| $B_{15}$ | Turkey | Kizildag | 36 21 | 35 58 | 400 |
| $B_{16}$ | Iraq | Zawita | 36 35 | 44 20 | 400 |

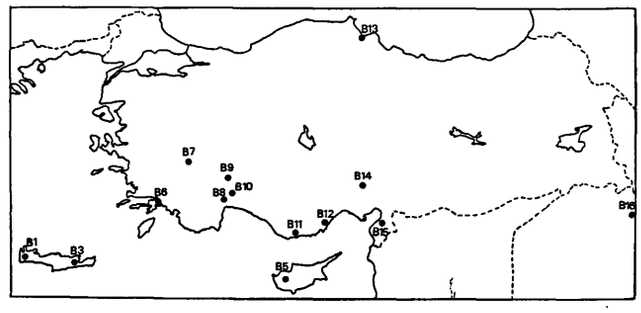


*Figure 1.* — Seed origins of *Pinus brutia* Ten. subsp. *brutia* in provenance trial plots.

*Table 1 bis.* — Israeli plantations of *Pinus brutia* subsp. *brutia* (lat. and long. according to Israeli Gird).

| Code | Locations | lat. N | long. E | Year(s) of planting |
|---|---|---|---|---|
| EL | Elon | 274 | 171 | 1940 |
| BF | Balfour Forest | 231 | 174 | 1933 |
| HZ | Hazorea | 227 | 161 | 1946 |
| ME | Mishmar haEmeq | 223 | 164 | 1949 |
| ES | En haShofet | 222 | 160 | 1947 |
| IL | Ilanot | 188 | 141 | 1950 |
| BS | Ben Shemen | 151 | 145 | 1958-1976 |
| MB | Mevo Betar | 126 | 160 | 1951-1972 |

In the text, graphs and tables, forest plantations are listed by the code and subscript (year of planting abbreviated by omitting the century), *e.g.* $BS_{58}$ stands for Ben Shemen plantation of 1958.

## Results

*Figure 2* shows a typical chromatogram of needle resin composition in *P. brutia* subsp. *brutia*. Of the many compounds (peaks) occurring in measurable amounts the following were identified by comparison with pure

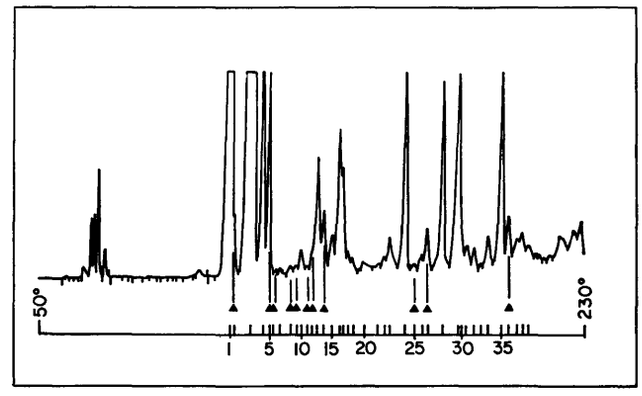


*Figure 2.* — Typical chromatograph of needle resin of *Pinus brutia* subsp. *brutia* (▲, compounds used in statistical analyses).

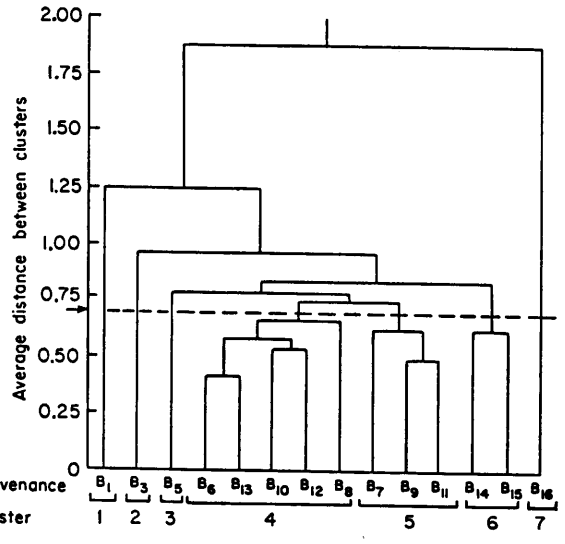


*Figure 3.* — Cluster analysis of seed origins of *Pinus brtia* subsp. *brutia* in provenance trials seed.

standards: peak 1 = $\alpha$-pinene; peak 2 = camphene; peak 3 = $\beta$-pinene; peak 4 = myrcene; and peak 5 = $\Delta$ 3 — carene.

Of the many compounds (peaks) on the chromatogram (*Fig. 2*) only 36 compounds which show variation in their relative (%) quantities were subjected to statistical analysis.

*1. Provenance trials*

Results of cluster analysis, using the average linkage method, of the 36 compounds (illustrated in the example of *Fig. 3*) show the arrangement of the provenances into seven major groups or clusters. Four groups consist each of a single seed source: low and high altitude provenances from Crete ($B_1$ and $B_3$, clusters 1 and 2), Cyprus ($B_5$, cluster 3), and Iraq ($B_{16}$, cluster 7). The remaining three groups are composed of Turkish seed sources: cluster 4 consists of provenances from a low elevation in the Taurus range ($B_6$, $B_8$, $B_{10}$, $B_{12}$) and the Black Sea coast ($B_{13}$); cluster 5 includes high-altitude provenances from the Taurus ($B_7$, $B_9$ and $B_{11}$); and cluster 6 consists of two seed sources from the Ala Mts. ($B_{14}$) and the Amanos Mts. ($B_{15}$).

*Table 2.* — Results of backward stepwise discriminant analysis (limits; $P < 0.05$; for peak numbers see chromatograph in *Figure 2*).

| Compound (peak No.) | F | Prob > F |
|---|---|---|
| 2 | 1.852 | 0.0374 |
| 5 | 2.622 | 0.0022 |
| 6 | 4.045 | 0.0001 |
| 8 | 2.458 | 0.0041 |
| 9 | 4.004 | 0.0001 |
| 11 | 2.373 | 0.0056 |
| 12 | 3.181 | 0.0002 |
| 14 | 3.247 | 0.0002 |
| 25 | 3.160 | 0.0003 |
| 27 | 1.929 | 0.0286 |
| 36 | 4.158 | 0.0001 |

Backward stepwise discriminant analysis procedure was implemented to reduce the number of variables (compounds), for further statistical analysis, from 36 to 11 by conserving only those which contribute significantly to the discrimination among the 14 provenances. Of the compounds selected, as indicated in *Figure 2.*, 4 compounds (2, 5, 14, 27, and 36) are major compounds whereas the others (6, 8, 9, 11, 12 and 25) are minor compounds.

*Table 3.* — Percentages of trees from each provenance assigned by discriminant analysis to each of the provenances (chemotypes).

| From provenanc | CLUSTER (Fig. 3) | Into Provenances (chemotypes) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B1 | B3 | B5 | B6 | B8 | B10 | B12 | B13 | B7 | B9 | B11 | B14 | B15 | B16 | n |
| B 1 | 1 | 55.6 | 11.1 | 0.0 | 0.0 | 0.0 | 0.0 | 11.1 | 0.0 | 0.0 | 11.1 | 0.0 | 0.0 | 0.0 | 11.1 | 9 |
| B 3 | 2 | 0.0 | 71.4 | 0.0 | 0.0 | 14.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.3 | 0.0 | 7 |
| B 5 | 3 | 4.2 | 4.2 | 20.8 | 8.3 | 4.2 | 4.2 | 12.4 | 0.0 | 20.8 | 8.3 | 4.2 | 4.2 | 4.2 | 0.0 | 24 |
| B 6 | 4 | 0.0 | 0.0 | 0.0 | 50.0 | 0.0 | 0.0 | 5.6 | 11.0 | 0.0 | 16.6 | 5.6 | 5.6 | 5.2 | 0.0 | 18 |
| B 8 | | 5.0 | 0.0 | 0.0 | 0.0 | 30.0 | 10.0 | 5.0 | 5.0 | 5.0 | 15.0 | 10.0 | 0.0 | 5.0 | 10.0 | 20 |
| B10 | | 0.0 | 11.1 | 5.6 | 0.0 | 11.1 | 22.1 | 5.6 | 0.0 | 5.6 | 11.1 | 11.1 | 5.6 | 11.1 | 0.0 | 18 |
| B12 | | 0.0 | 13.3 | 0.0 | 0.0 | 13.3 | 0.0 | 33.3 | 6.7 | 6.7 | 0.0 | 6.7 | 0.0 | 13.3 | 6.7 | 15 |
| B13 | | 0.0 | 4.5 | 4.5 | 18.2 | 9.1 | 0.0 | 4.5 | 18.2 | 4.5 | 13.7 | 9.1 | 4.5 | 13.7 | 0.0 | 22 |
| B 7 | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 7.1 | 7.1 | 0.0 | 0.0 | 50.0 | 28.5 | 0.0 | 7.1 | 0.0 | 0.0 | 14 |
| B 9 | | 0.0 | 0.0 | 0.0 | 12.5 | 6.2 | 0.0 | 6.2 | 0.0 | 18.8 | 56.3 | 0.0 | 0.0 | 0.0 | 0.0 | 16 |
| B11 | | 0.0 | 0.0 | 0.0 | 9.5 | 9.5 | 4.8 | 4.8 | 0.0 | 4.8 | 14.3 | 47.5 | 0.0 | 0.0 | 4.8 | 21 |
| B14 | 6 | 9.9 | 9.1 | 0.0 | 9.1 | 0.0 | 0.0 | 0.0 | 0.0 | 9.1 | 18.2 | 0.0 | 36.3 | 9.1 | 0.0 | 11 |
| B15 | | 0.0 | 12.5 | 8.3 | 8.3 | 8.3 | 4.2 | 8.3 | 0.0 | 4.2 | 0.0 | 0.0 | 8.3 | 37.6 | 0.0 | 24 |
| B16 | 7 | 0.0 | 0.0 | 0.0 | 15.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 84.6 | 13 |

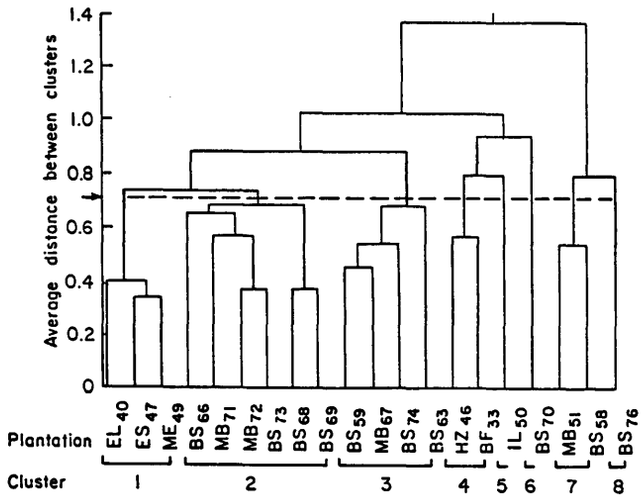


*Figure 4.* — Cluster analysis of *Pinus brutia* subsp. *brutia* in forest plantations.

The F values and probabilities of F are presented in *Table 2*. The squared canonical correlation of the set is 0.1288.

Each provenance is composed of an ensemble of chemotypes (genotypes) occurring at various rates. To evaluate the within-provenance diversity in resin composition discriminant analysis procedure using the set of the 11 variables presented in *table 2*, was imposed on each single tree resin composition. Since 14 provenances were used in this analysis, 14 discriminant functions were created by which the posterior probabilities of each tree to belong to a given provenance was calculated. The set of compound combinations which yield maximum probability to a given provenance is considered to represent its typical chemotype. Thus each of the 232 trees resin compositions was assigned to one of the chemotypes. The results present in *table 3*. Show that in each of the provenances more than one "chemotype" was identified. Only in 6 of the 14 provenances belonged more than 50% of the trees to the same chemotype; in 5 other provenances only between 30% to 50% of the trees were of the same chemotype. The trees of provenance $B_{16}$ are of 2 chemotypes, one of which is represented by 84% of the trees. The trees of provenance $B_3$ are of three chemotypes, one of which occurs at the rate of 71% of the trees. The trees of provenance $B_1$ consist of 5 chemotypes, one of which comprise 55% of the trees. Low elevation populations grouped in cluster 3, 4 and 6 are divided among relatively more chemotypes than the high elevation populations grouped in cluster 5.

*2. Artificial forest plantations*

To identify possible relations among 513 trees in 20 plantations in Israel (*Table 1 bis*) average linkage cluster analysis was made using the relative mean quantity (%) of the 36 compounds. Results present in *figure 4* show the grouping of the plantations into eight clusters. Plantations planted between 1933 and 1949 are grouped into two clusters (clusters 1 and 4); those established after 1949 are grouped into 6 clusters, with clusters 2 and 3 including most of the plantations.

Using the 14 discriminant functions, by which the probability of trees growing in the provenance trials to

*Table 4.* — Percentages of the trees from plantations and stands assigned by discriminant analysis to chemotypes from provenance trials.

| From Plantations and Stands | Clusters (Fig. 4) | Into Chemotypes from Provenance trials | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B1 | B3 | B5 | B6 | B8 | B10 | B12 | B13 | B7 | B9 | B11 | B14 | B15 | B16 | n |
| EL 40 | 1 | 56.7 | 3.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 26.7 | 6.7 | 3.3 | 3.3 | 0.0 | 30 |
| ES 47 | | 53.9 | 0.0 | 0.0 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 26.9 | 3.8 | 7.7 | 0.0 | 3.9 | 26 |
| ME 49 | | 55.6 | 0.0 | 0.0 | 3.7 | 3.7 | 0.0 | 0.0 | 0.0 | 7.4 | 22.2 | 3.7 | 0.0 | 3.7 | 0.0 | 27 |
| BS 66 | 2 | 54.6 | 0.0 | 0.0 | 3.0 | 0.0 | 0.0 | 18.2 | 0.0 | 3.0 | 0.0 | 3.0 | 9.1 | 9.1 | 0.0 | 33 |
| BS 68 | | 34.6 | 0.0 | 0.0 | 3.9 | 0.0 | 0.0 | 3.8 | 0.0 | 0.0 | 0.0 | 38.5 | 3.8 | 15.4 | 0.0 | 26 |
| BS 69 | | 42.2 | 0.0 | 0.0 | 11.6 | 0.0 | 0.0 | 11.6 | 0.0 | 11.6 | 0.0 | 7.5 | 11.6 | 3.9 | 0.0 | 26 |
| MB 71 | | 62.5 | 8.3 | 4.2 | 0.0 | 0.0 | 0.0 | 12.5 | 0.0 | 0.0 | 0.0 | 8.3 | 0.0 | 4.2 | 0.0 | 24 |
| MB 72 | | 50.0 | 0.0 | 3.6 | 0.0 | 0.0 | 0.0 | 3.6 | 0.0 | 0.0 | 7.1 | 7.1 | 10.7 | 17.9 | 0.0 | 28 |
| BS 73 | | 31.8 | 4.5 | 0.0 | 0.0 | 0.0 | 0.0 | 36.4 | 0.0 | 0.0 | 4.5 | 0.0 | 18.2 | 4.6 | 0.0 | 22 |
| BS 59 | 3 | 29.2 | 4.2 | 0.0 | 0.0 | 0.0 | 0.0 | 45.8 | 0.0 | 0.0 | 4.2 | 0.0 | 8.3 | 8.3 | 0.0 | 24 |
| BS 63 | | 26.0 | 0.0 | 3.7 | 0.0 | 0.0 | 0.0 | 33.3 | 0.0 | 3.7 | 22.2 | 3.7 | 3.7 | 3.7 | 0.0 | 27 |
| MB 67 | | 27.3 | 0.0 | 0.0 | 4.6 | 0.0 | 0.0 | 22.7 | 0.0 | 0.0 | 0.0 | 13.4 | 13.7 | 18.3 | 0.0 | 22 |
| BS 74 | | 13.0 | 0.0 | 4.4 | 4.4 | 0.0 | 4.4 | 13.0 | 0.0 | 13.0 | 4.4 | 8.7 | 21.7 | 13.0 | 0.0 | 23 |
| BF 33 | 4 | 91.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.9 | 0.0 | 0.0 | 2.9 | 0.0 | 0.0 | 0.0 | 2.9 | 34 |
| HZ 46 | | 82.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14.3 | 0.0 | 0.0 | 0.0 | 3.6 | 28 |
| IL 50 | 5 | 42.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.1 | 0.0 | 7.1 | 7.1 | 14.3 | 0.0 | 0.0 | 21.5 | 14 |
| BS 70 | 6 | 65.5 | 6.9 | 0.0 | 0.0 | 0.0 | 0.0 | 13.8 | 0.0 | 3.4 | 0.0 | 0.0 | 0.0 | 3.5 | 6.9 | 29 |
| MB 51 | 7 | 10.5 | 0.0 | 0.0 | 0.0 | 0.0 | 10.5 | 26.3 | 0.0 | 21.0 | 26.3 | 0.0 | 0.0 | 5.4 | 0.0 | 19 |
| BS 58 | | 10.7 | 0.0 | 3.6 | 17.9 | 0.0 | 0.0 | 57.1 | 0.0 | 0.0 | 0.0 | 7.1 | 0.0 | 3.6 | 0.0 | 28 |
| BS 76 | 8 | 0.0 | 3.7 | 3.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 48.2 | 0.0 | 11.1 | 33.3 | 0.0 | 27 |

belong to each one of the chemotypes was calculated, discriminant analysis was imposed on the 517 trees in 20 planted stands (*Table 1 bis*) to determine their chemotypes. Results are presented in *table 4*. Whereas certain chemotypes are of very rare occurrence, others are rather common. Except for one stand ($BS_{76}$), all stands contain the $B_1$ chemotype though in various amounts. Common chemotypes in many plantations are: $B_9$, $B_{11}$, $B_{12}$, $B_{14}$ and $B_{15}$; the rare occurrence of the chemotypes $B_3$, $B_5$, $B_6$, $B_7$, $B_8$, $B_{10}$, $B_{16}$, and the complete absence of the $B_{13}$ chemotype, are noteworthy. In plantations grouped in cluster 1, the $B_1$ and $B_9$ chemotypes are the main ones, with only small amounts of the others. The composition of plantations grouped in clusters 2 and 3 differs only slightly. Plantations in cluster 2 have higher frequencies of the $B_1$ chemotype than those grouped in cluster 3. Plantations grouped in cluster 4 have in common only three chemotypes, of them $B_1$ in very high frequencies, whereas plantations grouped in cluster 7 contain very low frequencies of the $B_1$ chemotype.

**Discussion and Conclusions**

As presently understood (NAHAL, 1983), *P. brutia* is composed of four subspecies: subs. *brutia,* the subject of this paper; subsp. *stankewiczii* (SUKACZEW) NAHAL; subsp. *pithyusa* (STEVENSON) NAHAL; and subsp. *eldarica (*MEDW.) NAHAL, which may include the so-called Afghan or Quetta pine. Owing to their narrow endemism in the Crimea, the northeastern Black Sea coast and Mt. Eldar in Transcaucasia, respectively, variation of the last 3 taxa may by expected to be small. In conrast, much genetic diversiy is likely to occur in subsp. *brutia* due to its large range (CRITCHFIELD and LITTLE, 1966). Several recent papers (CALAMASSI *et al.,* 1988 a and b; CONKLE *et al.,* 1988; FALUSI, 1982; FALUSI and CALAMASSI, 1982; ISIK, 1986; PALMBERG, 1975: PANETSOS, 1981; SCHILLER and GRUNWALD, 1987; SPENCER, 1985) have described the occurrence of variation as related to seed origin. Variation within seed sources growing in the provenance trial plots, were found by WEINSTEIN (1982, 1988).

As shown in this study as well as in a preceding paper (SCHILLER and GRUNWALD, 1987), resin composition, be it of needles or cortex, can discriminate among provenances, quantify differences and estimate affinities among various seed sources of subsp. *brutia.* Cluster analysis of the needle resin composition (*Fig. 3*) provides results fairly similar to those of cortex resin composition of the same provenances (SCHILLER and GRUNWALD, 1987). In both clusters the low-altitude provenance from Crete ($B_1$) and the Iraqi provenance ($B_{16}$) are placed at the extremes of the cluster, with the high-altitude provenance from Crete ($B_3$) now in a different cluster which splits off early from the others. Provenance $B_{13}$ from the Black Sea coast of Anatolia now shifts away from the Ala range ($B_{14}$) and Amanos Mts. ($B_{15}$) provenances to join the large group of low lying seed sources of western Anatolia and the Taurus range. There are other minor changes in *Figure 3* compared with the clustering in an earlier study (SCHILLER and GRUNWALD, 1987), but there is little to gain by further discussion of differences from earlier formed clusters, since variation in different characters — in our case needle and cortex terpene composition — is not necessarily parallel.

The distinct position in the cluster of provenances $B_{14}$ and $B_{15}$ confirms the division of subsp. *brutia* into a western and eastern 'group' postulatet earlier (CONKLE *et al.,* 1988). As now understood, the divide along approximately the 35° E meridian in the southern Mediterranean area of the subspecies can be related to orographic factors, the western race of western Anatolia and the Lycian and Cilician Taurus being separated from the eastern race of the eastern Taurus (Ala Mts.) and its outlying offshoot of the Amanos Mts. by the Cilician gates ,the high Bolkar range (alt. 3585 m) and the coastal plain of Cilicia.

As mentioned earlier, the objective of our investigation was to identify if possible, the seed sources of stands in forest plantations by comparing the frequencies of chemotypes occurring in the planted stands with those occurring in provenance trials grown from well documented seed origins. With hindsight it is, however, unlikely that a complete match could be found: First, because of the dispersion within provenances which resulted in incomplete match even in table 3 with known sources; second, chemotypes obtained from needle resin may have limited discriminant power; and least, the absence in provenance trials of seed sources from continental Greece and the Aegen Greek islands, since Greece figures prominently as an actual seed supplier, and with but two seed sources from Crete (*Table 1. Figure 1)* leaves this part of the area of brutia pine manifestly under-represented. The same applies with regard to Cyprus, where genetic variation with altitude is most likely (CONKLE *et al.,* unpublished data), since subsp. *brutia* occurs naturally from almost sea level to approximately 1500 m above sea level (ISIK, 1986). In addition, the latitude and altitude quoted by the seed collectors (*Table 1*) show that the single seed source from Cyprus is from an artificial plantation of unknown origin in the Mesaoria near Nicosia airport and therefore does not describe the genetic make-up of *Pinus brutia* on the island.

Let us now see how far we can go in estimating the seed origin of stands in Israel forest plantations of brutia pine. The fact that with one exception, the $B_1$ chemotype, most common in the $B_1$ Cretean provenance, occurs in all the plantations examined (*Table 4*), hardly facilitates our objective, since it is unlikely that a significant proportion of the seed imported and used here for planting did originate from Crete. It would be tempting to speculate that this chemotype is typical of provenances from low levels in the Greek islands, though admittedly there is so far but little supporting evidence for this assumption.

The earliest plantations examined which could only have been established from imported seed, display high frequencies of the $B_1$ chemotype and lower ones of the $B_7$, $B_9$, $B_{10}$ and $B_{16}$ chemotypes (*Table 4*) and, more significantly, are grouped into three clusters: cluster 1 ($EL_{40}$, $EH_{47}$ and $ME_{49}$), cluster 4 ($BF_{33}$ and $HZ_{46}$), and cluster 5 ($IL_{50}$) (*Table 4, Figure 4*). We therefore speculate that the seed used for establishment of these stands was brought from unidentified parts of Greece, the more as we know for certain that the seed of the Ilanot plantation ($IL_{50}$) did indeed originate from mainland Greece where hybridization between *P. halepensis* and probably planted brutia pine does exist (CONKLE et al., unpublished data; PANETSOS, 1975, 1986).

With regard to the plantations established in the 1960s and 1970s, there is evidence of both imports and local collections (Y. REVES, personal communication). Use of local seed could further complicate our work because of cross-pollination among Aleppo and brutia pines and among different provenances of brutia pine planted close

to the seed trees. Cross pollination might account for the occurrence of the $B_1$ chemotype also in locally collected seed and not only in Greek seed material.

The stands grouped into clusters 2, 3 and 7 have in common relatively high frequencies of chemotypes that occur in low-altitude Turkish provenances and could therefore well be from imported Turkish seed. The occurrence at relatively low frequencies of the $B_1$ chemotype emphasizes the possibility that these stands are the product of a mixture of Turkish seed with Greek or local seed collected in stands pollinated with Aleppo pine. The single plantations in clusters 6 and 8 appear to be from Greek and Turkish seed, respectively; a likely seed source for $BS_{76}$ is the higher elevation of the Taurus (*Table 4*). Whereas the rarity in local plantations of chemotypes from the Lycian Taurus ($B_8$, $B_{10}$) and the absence of the Black Sea coast chemotype $B_{13}$ points out clearly that no seed collected there ever reached Israel.

In spite of the fact that throughout the years Cyprus was an important seed supply source (Y. REVES, personal communication), the conspicuously low rate of the Cyprus chemotype $B_5$ in the plantations examined (*Table 4*) is doubtlessly due to the character of the population purporting to represent the island in the provenance trials, the very wide spectra of chemotypes (*Table 3*) being apparently atypical of natural stands.

In conclusion, analysis of needle resin composition in *P. brutia* subsp. *brutia* could probably be applied successfully to determine with a reasonable degree of approximation the origin of seed used in plantation establishment, provided some conditions are fulfilled. First, there is need for a relatively dense network of reference points (seed sources) in provenance trials to cover the entire natural range of brutia pine, with special emphasis on the study of genetic diversity along altitudinal clines and in particular habitats such as outliers and islands with a long history of isolation. Second, care must be taken in the selection of seed sources to be included in provenance trials, with planted stands (or natural regeneration from such stands) to be strictly rejected, if not expressly recorded as such. The only definite conclusions which can be drawn from this study are that: (i) There is a lack of a sufficiently dense and wideranging network of seed source for this investigation to produce reliable results; (ii) the native character of at least one provenance included in the provenance trials is questionable; and (iii) a wide margin of error exist in the estimates of the seed origin of the plantations investigated.


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# Monte Carlo Simulation Models of Breeding-Population Advancement


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### Summary

Five generations of population improvement were modelled using Monte Carlo simulations. The model was designed to address questions that are important to the



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